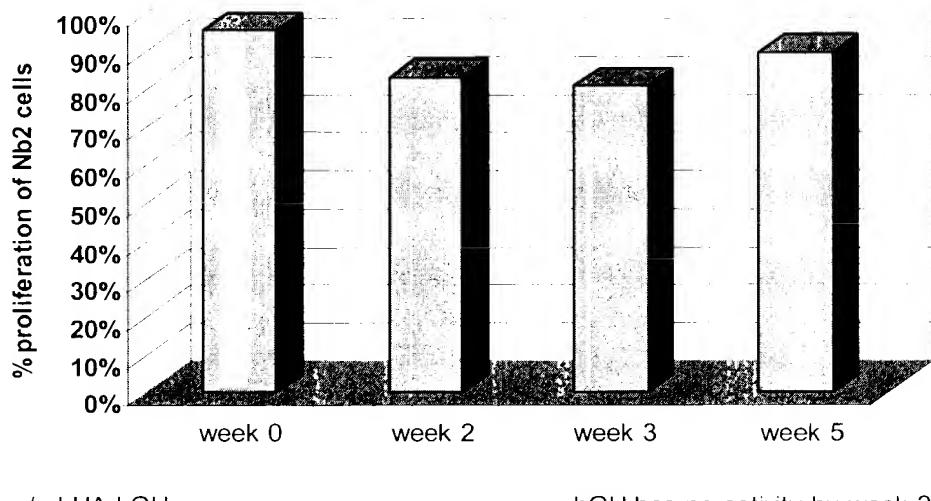


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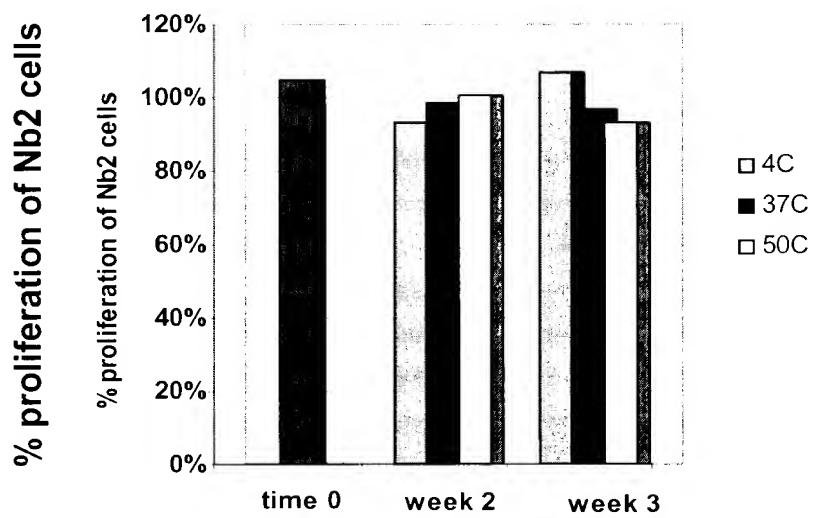
**Stability of HA-hGH at 37°C in cell culture media**



**Figure 1**

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**Stability of HA-hGH in cell culture media**

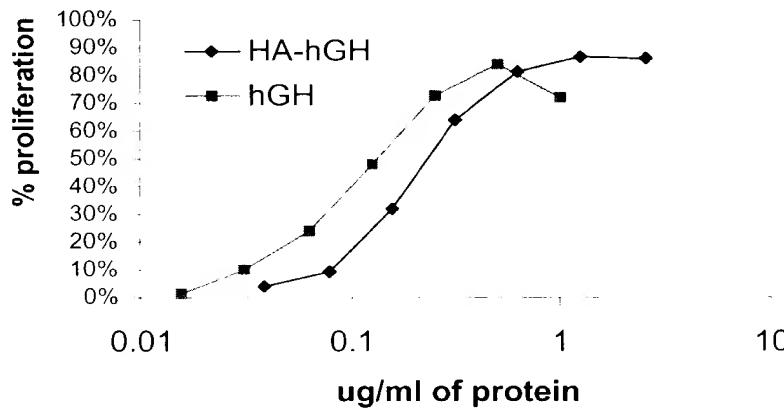


60ng/ml of HA-hGH

**Figure 2**

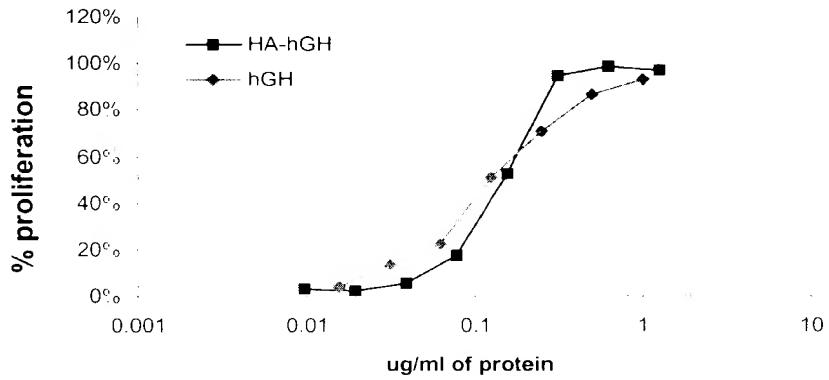
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**Nb2 Cell Proliferation Assay (24hrs)**



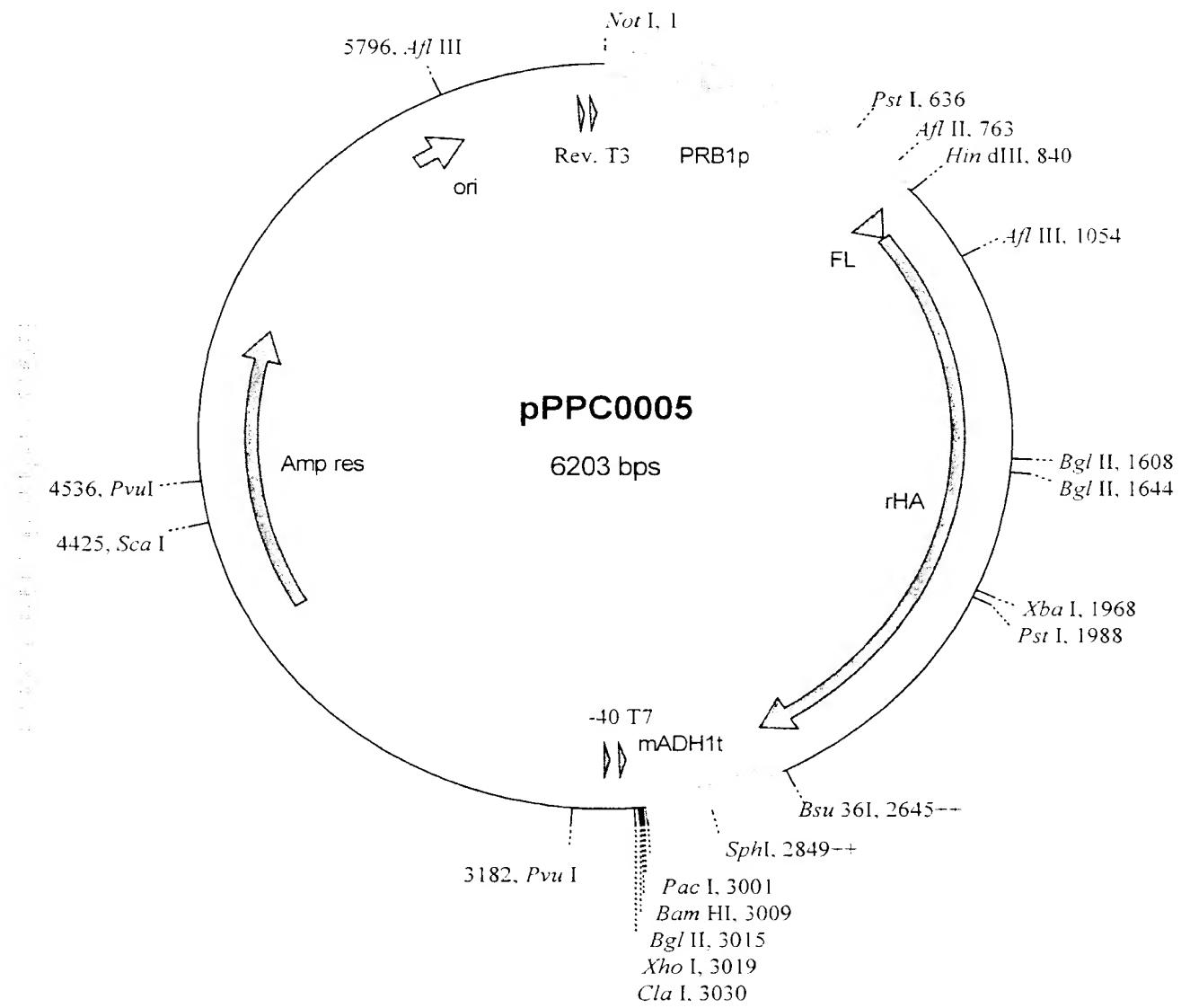
**Figure 3A**

**Nb2 Cell Proliferation Assay (48hrs)**



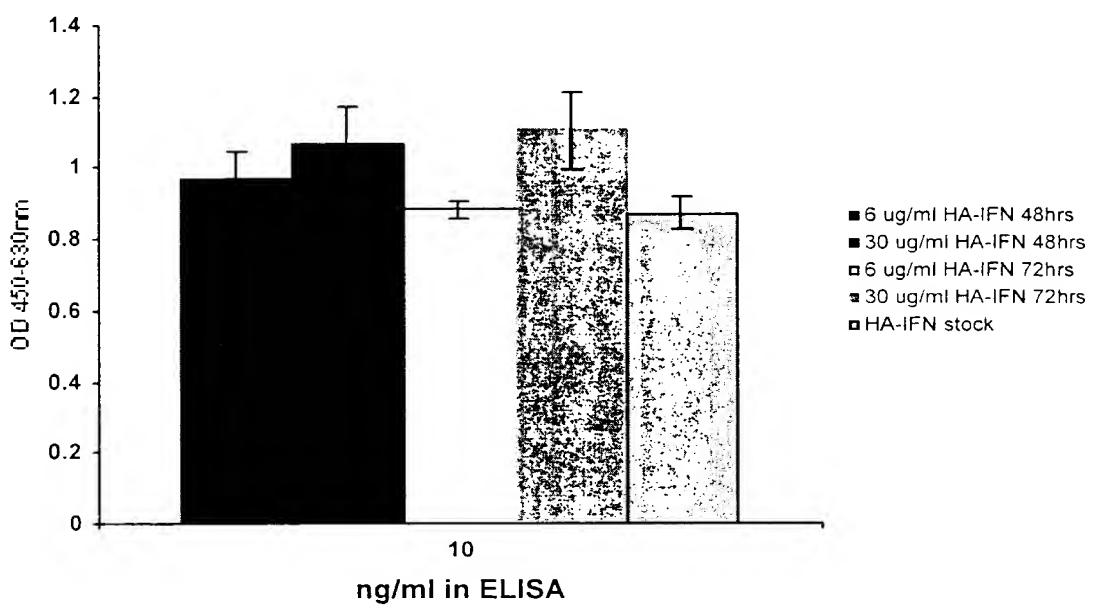
**Figure 3B**

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**Figure 4**

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**Figure 5**

Figure 6

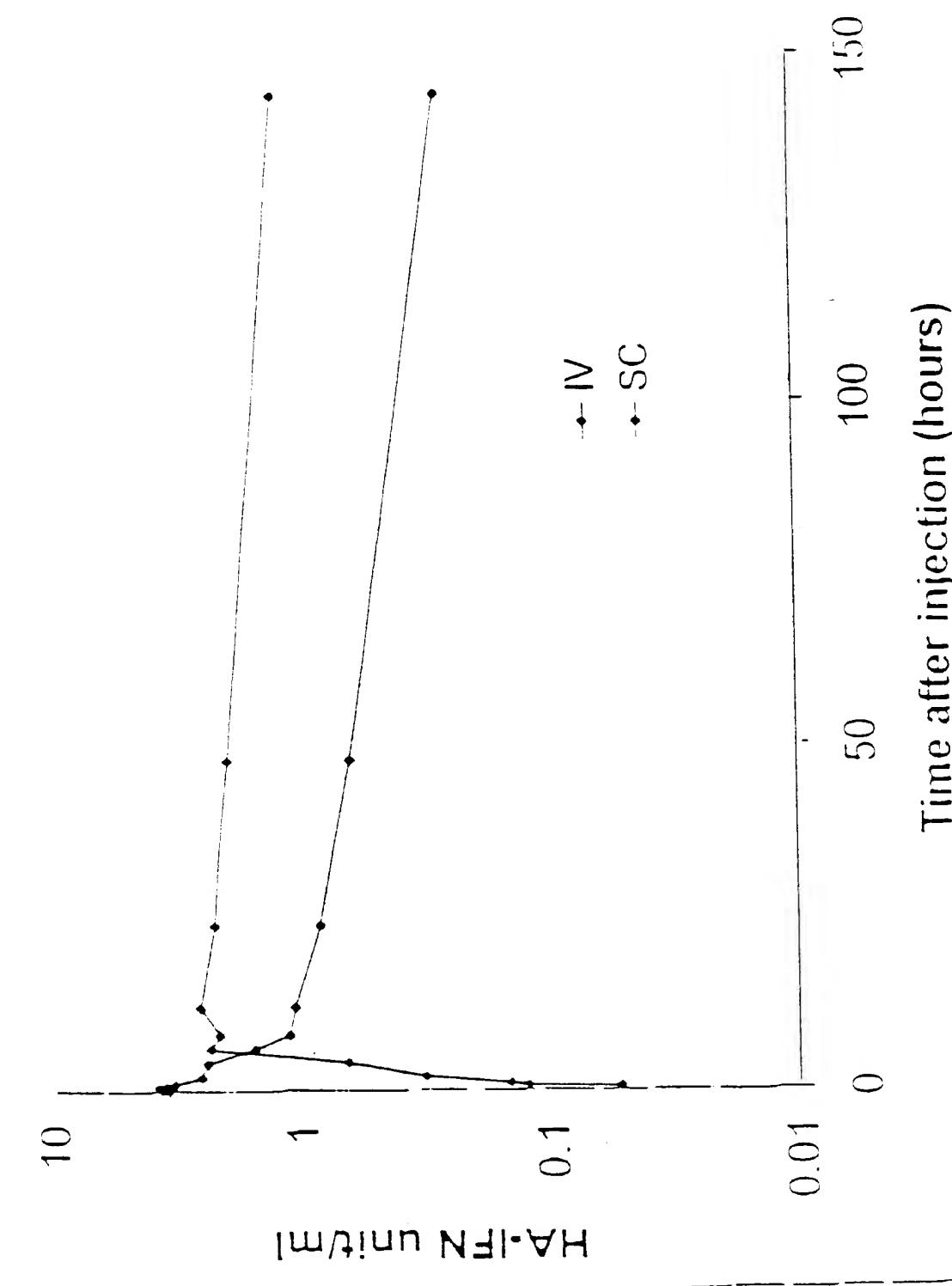
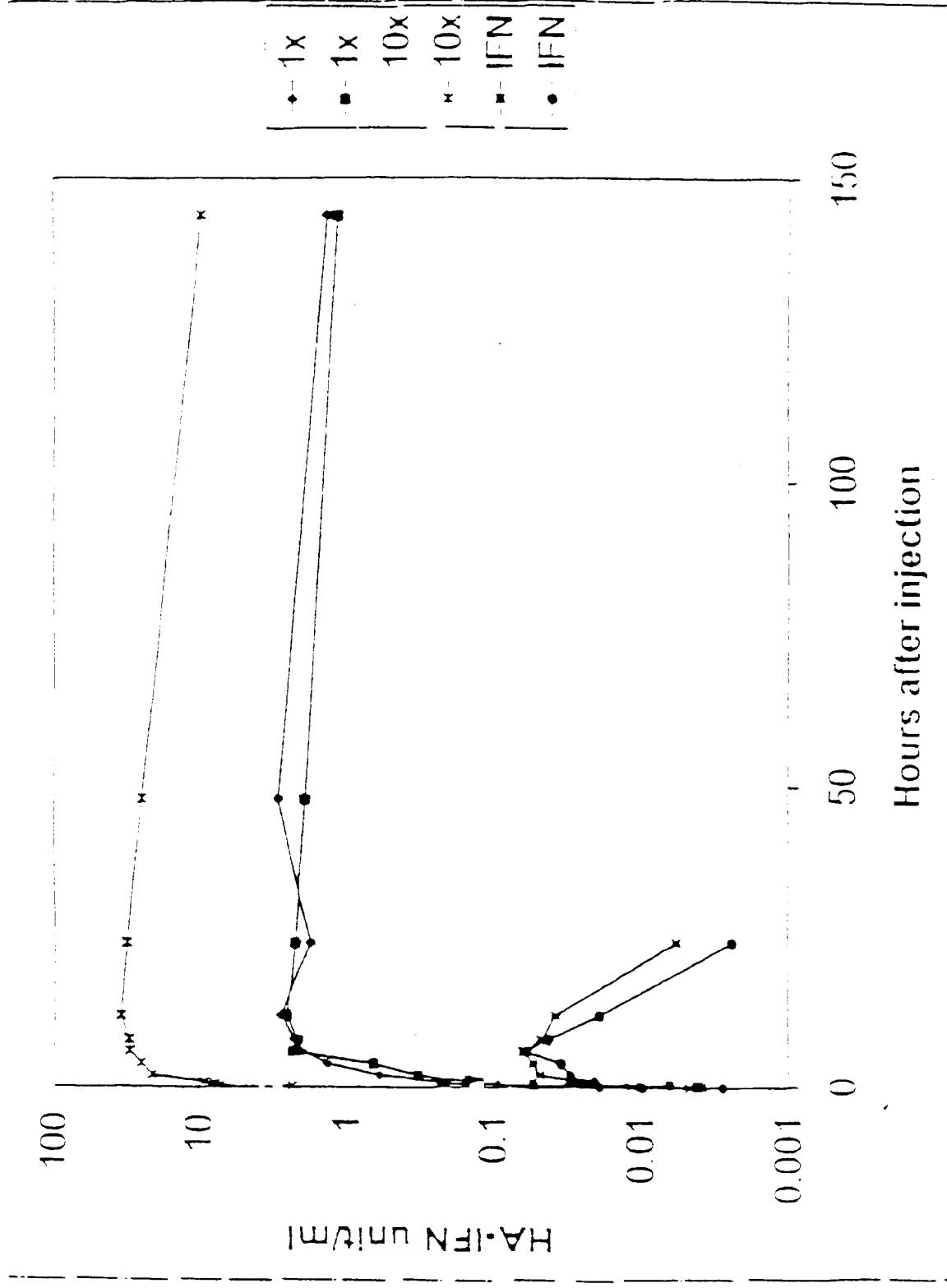
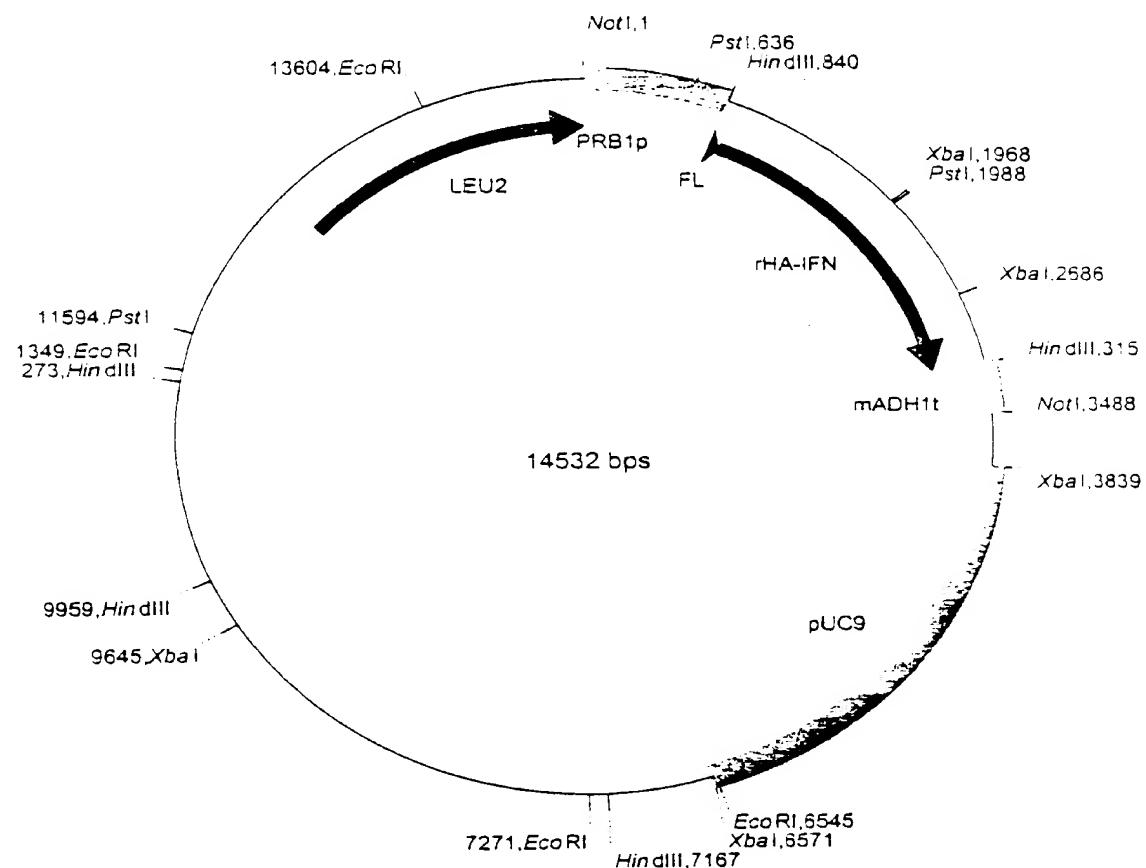


Figure 7





**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises  
*PRB1* promoter, from *S. cerevisiae*.  
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MIF $\alpha$ -1 leader.  
 HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)  
*ADH1* terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III/BamHI* fragment generally used.

**Figure 8**

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## Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

1 DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA  
       HHHHH HHH       HHH HHHHHHHHHHH                   HHHHH HHHHHHHHHHH  
       I                   II                   III  
 51 KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE  
       HHHHH           HHHHH HHHHH           HHHH H       HHHH  
 101 CFLQHKDDNP NLPPPLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY  
       HHHH           H HHHHHHHHH           HHHHHHHHHH HHHHH  
       IV  
 151 APELLFFAKR YKAAPTECCO AADKAACLLP KLD~~E~~LRDEGK ASSAKQLKC  
       HHHHHHHHHH HHHHHHHHH           HHHHH HHHEHHHHHH HHHHHHHHH  
       V  
 201 ASLQKFGERA FKA~~WA~~VARLS QRFPKA~~E~~FAE VSKLVTDLTK V~~H~~TECCCHGDL  
       HHHHH       HH HHHHHHHHHHH HH           HHH HHHHHHHHHHH HHHHHH HH  
       VI                   VII  
 251 LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA  
       HHHHHHHHHH HHHHH           HHHHH           HHHHHHH H  
 301 DLPSLAADFV E~~S~~DVCKNYA EAKDVFLGMF LYEYAERHPD YSVVLLRLA  
       HHHH           HHHHH           HHHHHHH HHHHHH           HHHHHHHH  
       VIII  
 351 KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NC~~E~~LF~~E~~QLGE  
       HHHHHHHHHH           HH       H       HHHHH HHHHHHHHHHH HHHHHHH  
       IX  
 401 YKFQNALLVR YT~~K~~KVPQVST PTLVEVSRLN GKVGSWCCKH PEAKRMPCAE  
       HHHHHHHHHH HHHH       H HHHHHHHHHHH           HHH           HHHHHHHH  
       X                   XI  
 451 DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDET~~Y~~VPK  
       HHHHHHHHHH HHHHH           HHHHHHHHHH           HHHHHHHH  
 501 EFNAETFTFH ADICTLSEKE RQIKKQTALV ELV~~X~~HKPKAT KEQLKAVMDD  
       HHH       HHH HHHHHMMEEHHH HHH           HHHHHHHH  
       XII  
 551 FA~~A~~FVEKCCK ADDKETCFAE EGK~~K~~LVAASQ AALGL  
       HHHHHHHHH           HHHH HHHHHHHHHHH HH

| <b>Loop</b>      | <b>Loop</b>        |
|------------------|--------------------|
| I Val54-Asn61    | VII Glu280-His288  |
| II Thr76-Asp89   | VIII Ala362-Glu368 |
| III Ala92-Glu100 | IX Lys439-Pro447   |
| IV Gln170-Ala176 | X Val452-Lys475    |
| V His247-Glu252  | XI Thr478-Pro486   |
| VI Glu266-Glu277 | XII Lys560-Thr566  |

**Figure 9**

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

IV

151 APELLFFAKR YKAATTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

**X** represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

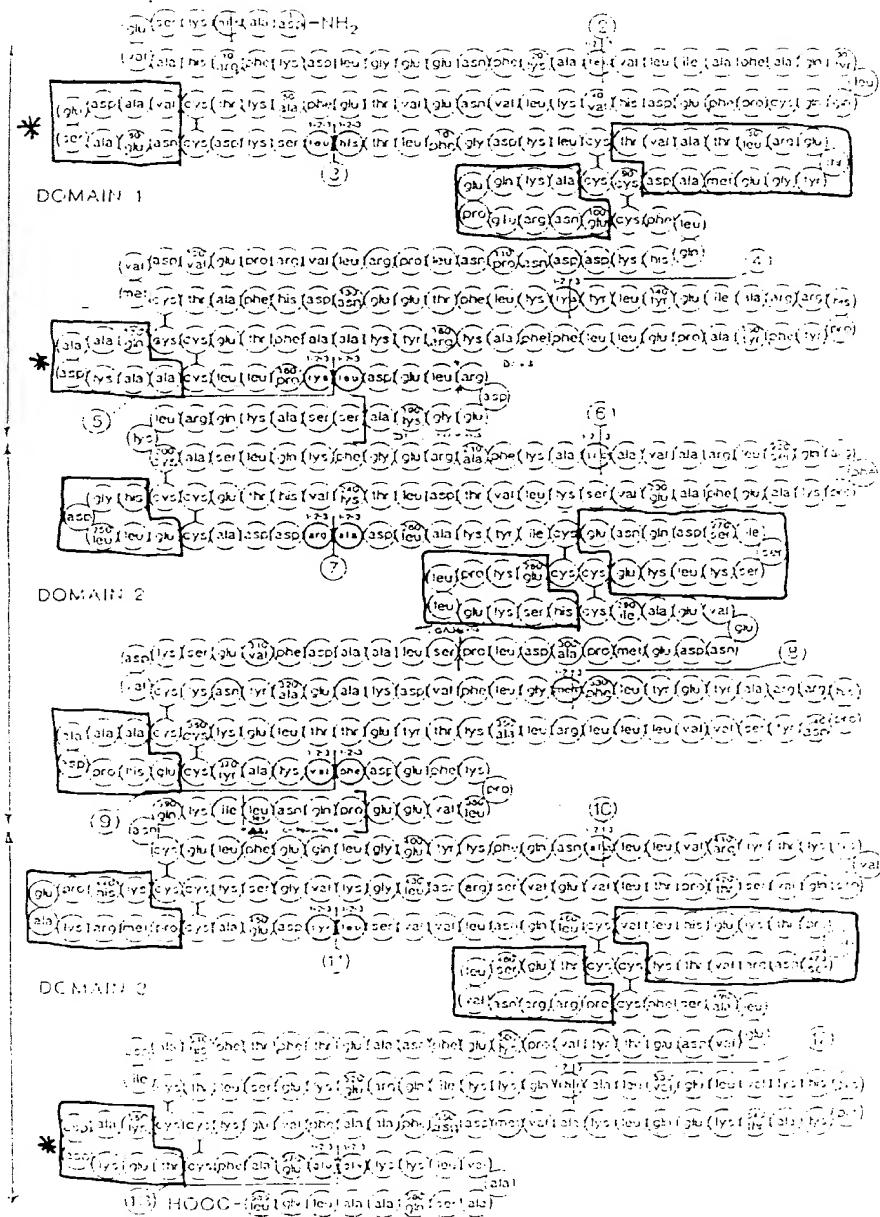
**b. Insertion (or replacement) of Randomised sequence into Loop IV.**

$(X)_n$   
 ↓  
 IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

**Figure 10**



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Disulfide bonds shown in yellow

**Figure 12: Loop IV Gln170-Ala176**

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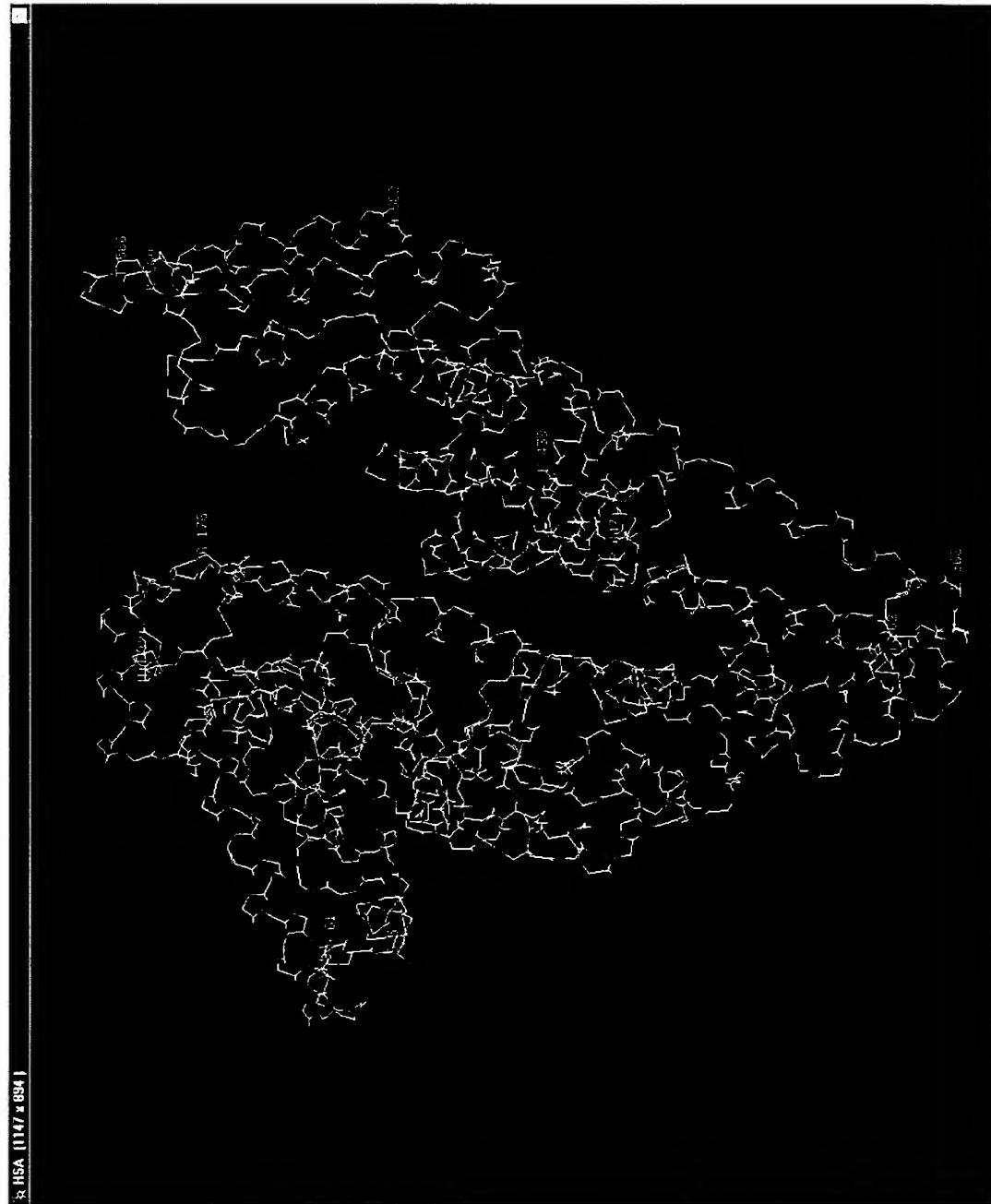
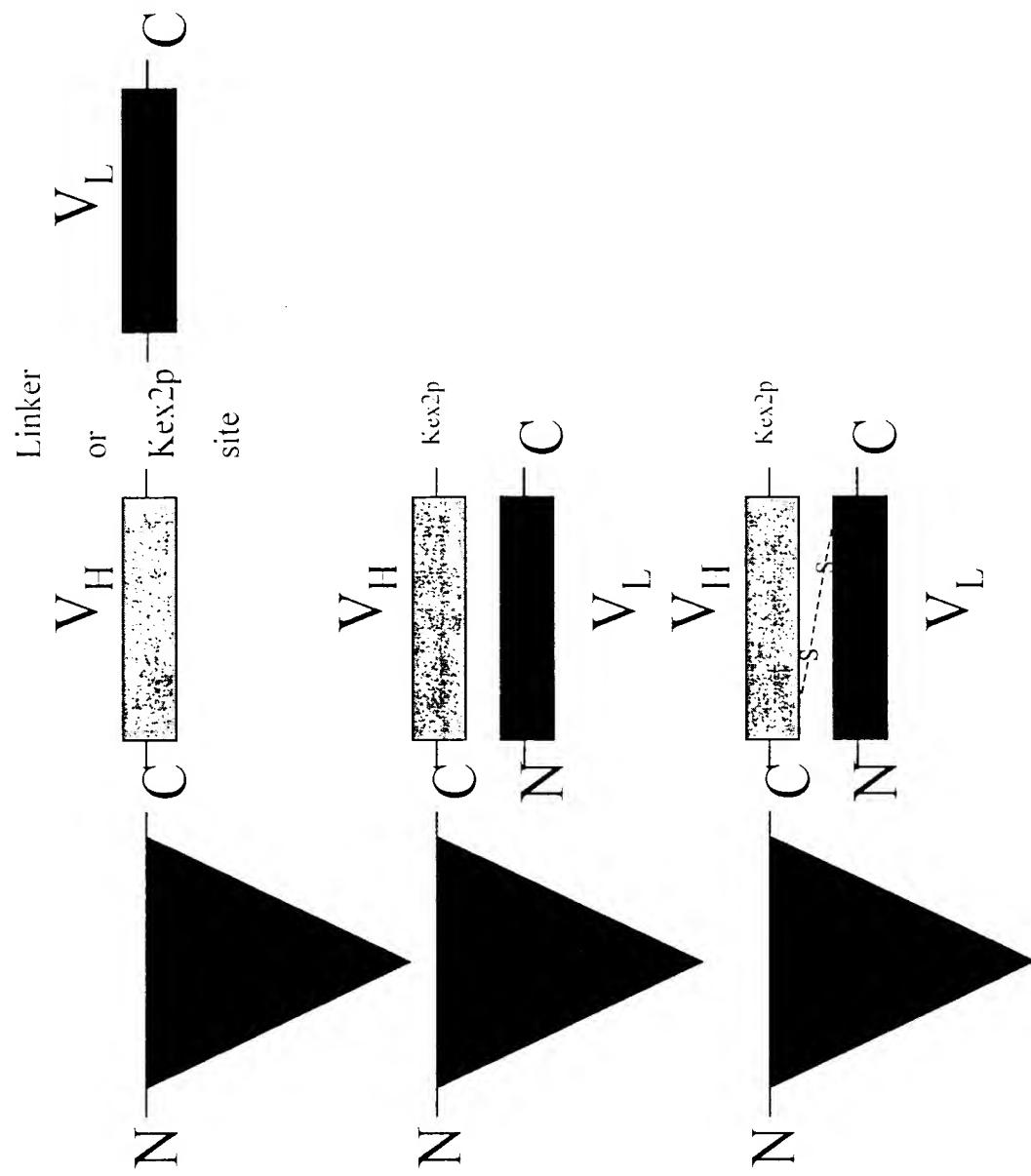


Figure 13: Tertiary Structure of HA



**Figure 14:** Schematic Diagram of Possible ScFv Fusions  
(Example is of a C-terminal fusion to HA)

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TGT GGA GAA GAT TTC AAA 60  
1 D A H K S E V A H R F K D L G E N F K 20

61 GCC TGT GTG TGT ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120  
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GAA ACT GAA TTT GCA AAA ACC TGT GTT CCT GAT GAG TCA GCT GAA 180  
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTC ACT CTT 240  
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360  
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGT ACT CTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
121 D V M C T A F H D N E E T F L K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAR GCC CGG GAA CTC CTT TTC TTT GCT AAA AGG 480  
141 E I A R R H P Y A P E L I F A K R R 160

Figure 15A

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481 TAT AAA GCT GCT TTT ACA GAA TGT GCT GAT AAA GCT GCT GGC TGC CTC TGT GAA 540  
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA C<sup>r</sup>PT C<sup>r</sup>GG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600  
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA GCA TGT GCA GTG G<sup>r</sup>TC CGC C<sup>r</sup>TG AGC 660  
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA G<sup>r</sup>CT GAA GAA G<sup>r</sup>TP T<sup>r</sup>C AA<sup>r</sup> G<sup>r</sup>TA G<sup>r</sup>CA GAT C<sup>r</sup>TT ACC AAA 720  
221 Q R F P K A E F A E V S K L V T D L T K 240

721 G<sup>r</sup>IC CAC AC<sup>r</sup> GAA T<sup>r</sup>G T<sup>r</sup>C CAT G<sup>r</sup>GA GAT C<sup>r</sup>TG C<sup>r</sup>TT GAA TGT GCT GAT GAC A<sup>r</sup>GG GCG GAC C<sup>r</sup>TT 780  
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840  
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900  
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GAA AGT AAG GAT G<sup>r</sup>T G<sup>r</sup>C AAA AAC TAT GCT 960  
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

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961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020  
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080  
341 Y S V L L R L A K T Y E T T L E K C 360  
361 C A A D P H E C Y A K V F D E F K P L 380

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140  
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1141 GTG GAA GAG GAG CTT CAG AAA TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200  
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCA CCC CAA GTG TCA ACT 1260  
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCT ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GCC AGC AAA TGT TGT AAA CAT 1320  
421 P T L V E V S R N L G K V G S K C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380  
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TGT CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440  
461 C V L H E K T P V S D R V T K C C T E S 480

**Figure 15C**

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|      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|------|
| 1441 | TTC | GTC | AAC | AGG | CBA | CCA | TTC | TTT | TCA | GCT | CAG | GAA | GTC | GAT | GAA  | AAG | TAC | GTC | CCC | AAA  | 1500 |
| 481  | L   | V   | N   | R   | R   | P   | C   | F   | S   | A   | L   | E   | V   | D   | E    | T   | Y   | V   | P   | K    | 500  |
| 1501 | GAG | TTT | AAT | GCT | GAA | ACA | TTC | ACC | TTC | CAT | GCA | GAT | ATA | TGC | ACA  | CCT | TCT | GAG | AAG | GAG  | 1560 |
| 501  | E   | F   | N   | A   | E   | T   | F   | T   | F   | H   | A   | D   | I   | C   | T    | L   | S   | E   | K   | E    | 520  |
| 1561 | AGA | CAA | ATC | AAG | AAA | CAA | ACT | GCA | CTT | GTC | CTT | GTC | AAA | CAC | AAG  | CCC | AAG | GCA | ACA | 1620 |      |
| 521  | R   | Q   | I   | K   | K   | Q   | T   | A   | L   | V   | E   | L   | V   | K   | H    | K   | P   | K   | A   | T    | 540  |
| 1621 | AAA | GAG | CAA | CAG | AAA | GCT | GTC | ATG | GAT | GAT | TTC | GCA | GCT | TTT | GTA  | GAG | AAG | TGC | TGC | AAG  | 1680 |
| 541  | K   | E   | Q   | L   | K   | A   | V   | M   | D   | D   | F   | A   | A   | F   | V    | E   | K   | C   | C   | K    | 560  |
| 1681 | GCT | GAC | GAT | AAG | GAG | ACC | TGC | TTT | GCC | GAG | GAG | GGT | AAA | AAA | CCT  | GTT | GCT | GCA | AGT | CAA  | 1740 |
| 561  | A   | D   | K   | E   | T   | C   | F   | A   | E   | E   | G   | K   | K   | L   | V    | A   | A   | S   | Q   | 580  |      |
| 1741 | GCT | GCC | TTA | GGC | TTA | TAA | CAT | CTA | CAT | TTA | AAA | GCA | TCT | CAG | 1782 |     |     |     |     |      |      |
| 581  | A   | A   | L   | G   | L   | *   |     |     |     |     |     |     |     |     |      |     |     |     |     | 585  |      |

Figure 15D